520 Rec'd PCT/PTO 11 FEB 2002

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER P61950US1
		US APPLICATION NO. (If known, see 37 CFR 1.5) 09/485473
INTERNATIONAL APPLICATION NO. PCT/EP98/05127	INTERNATIONAL FILING DATE 11 August 1998	PRIORITY DATE CLAIMED 11 August 1997
TITLE OF INVENTION	NEUTRAL SPHINGOMYELINASE	
APPLICANT(S) FOR DO/EO/US	Wilhelm STOFFEL, Kay HOFMANN and Stephan TOMIUK	

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    International Search Report — EPO
    PCT/IB/301 Form
    PCT/IB/304 Form
    PCT/IB/308 Form
    First Page of Publication
    International Preliminary Examination Report — with Annexes in German

US APPLICATION NO.(If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
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	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) . . $670.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . $760.00		
Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO) **$970.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00		
Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) **$840.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of $130.00 for furnishing the **oath or declaration** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	**Number Filed**	**Number Extra**	Rate		
Total Claims	**27** - 20 =	-7-	x $18.00	$ 126.00	
Independent Claims	**2** - 3 =	-0-	x $78.00	$	
Multiple Dependent Claim(s) (if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 966.00	
Reduction by 1/2 for filing by **small entity**, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 966.00	
Processing fee of $130 for furnishing the **English translation** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(f))				$	
TOTAL NATIONAL FEE =				$ 966.00	
Fee of $40.00 for recording the enclosed **assignment** (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				$	
TOTAL FEES ENCLOSED =				$ 966.00	
				Amt. to be refunded:	$
				Amt. charged:	$

a. ☒ A check in the amount of $ 966.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. **06-1358** in the amount of $ --- to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. **06-1358**. A duplicate copy of this sheet is enclosed.

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Atty. Dkt. No. P61950US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: STOFFEL, et al.

Serial No.: PCT/EP98/05127

Filed: 11 August 1998

For: NEUTRAL SPHINGOMYELINASE

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to calculating the filing fee, please amend the captioned application as follows.

IN THE CLAIMS

In claim 3, line 2, delete 'or 2".

In claim 5, line 1, change "at least one of claims 3 to 4" to --claim 3--.

In claim 7, line 2, change "at least one of claims 3 to 6" to --claim 3--.

In claim 8, line 1, change "at least one of claims 3 to 7" to --claim 3--.

In claim 5, line 1, change "at least one of claims 3 to 4" to --claim 3--.

In claim 10, line 2, delete "or 2".

In claim 12, line 2, delete "or 2".

In claim 20, line 2, change "any of claims 1 or 2" to --claim 1--.

In claim 21, lines 1-2, change "at least one of claims 3 to 8" to --claim 3--.

Rewrite the following claims.

9 (amended). Antibodies, characterized by being directed against the eucaryotic neutral sphingomyelinase according to [any of claims] claim 1 [or 2 or a nucleic acid according to at least one of claims 3 to 8].

14 (amended). A medicament containing the eucaryotic neutral sphingomyelinase according to [any of claims] claim 1 [or 2, a nucleic acid according to at least one of claims 3 to 8, and/or an antibody according to claim 9], together with further auxiliary agents

15 (amended). A diagnostic agent containing the eucaryotic neutral sphingomyelinase according to [any of claims] claim 1 [or 2, a nucleic acid according to at least one of claims 3 to 8, and/or an antibody according to claim 9], together with further auxiliary agents.

16 (amended). Use of the medicaments according to claim 14 [or the diagnostic agents according to claim 15] for the [diagnosis and] treatment of diseases based on over- or underexpression and/or an increased or reduced activity of eucaryotic neutral sphingomyelinase and/or disorders of cell proliferation, cell differentiation and/or apoptosis.

Add the following claims.

22. Antibodies, characterized by being directed against a nucleic acid according to claim 3.

23. A medicament containing a nucleic acid according to claim 3 together with further auxiliary agents.

24. A diagnostic agent containing a nucleic acid according to claim 3 together with further auxiliary agents.

25. A medicament containing an antibody according to claim 9 together with further auxiliary agents.

26. A diagnostic agent containing an antibody according to claim 9 together with further auxiliary agents.

27. Use of the diagnostic agents according to claim 15 for the diagnosis of diseases based on over- or underexpression and/or an increased or reduced activity of eucaryotic neutral sphingomyelinase and/or disorders of cell proliferation, cell differentiation and/or apoptosis.

REMARKS

The present claims are 1-27. By the instant amendment multiple dependencies are eliminated from the claims, thereby, reducing fees.

Favorable action is requested.

Respectfully submitted,

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Neutral Sphingomyelinase

The present invention relates to nucleic acids coding for eukaryotic neutral sphingomyelinase, and applications thereof.

Sphingomyelin is an essential component of plasma membranes. Degradation of sphingomyelin gives a number of substances having potential second messenger properties, e.g., ceramide, sphingosine, sphingosine-1-phosphate. Two sphingomyelin-cleaving enzymatic activities are known, namely that of lysosomal acid sphingomyelinase, and that of plasma-bound neutral sphingomyelinase.

Bacterial neutral sphingomyelinase is a secreted soluble protein.

The present invention for the first time provides nucleic acids coding for eukaryotic neutral sphingomyelinase. Eukaryotic neutral sphingomyelinase (nSMase) is characterized in that it cleaves sphingomyelin into ceramide and phosphocholine and that its activity depends on the addition of magnesium ions. It is a membrane-bound enzyme. Its maximum activity is achieved in the neutral pH range.

Figure 1 shows the gene sequence of human neutral sphingomyelinase.

Figure 2 shows the gene sequence of murine neutral sphingomyelinase.

Figure 3 shows the results of the Northern and Western blotting of nSMase-overexpressing cell lines.

Figure 4 shows the strategy for producing murine knockout mutants. The letters designate restriction sites.

Figure 5 shows constructs for obtaining transgenic mouse mutants.

Preferably, the nucleic acid according to the invention is a nucleic acid coding for the neutral sphingomyelinase of a mammal. More preferably, it codes for human or murine neutral sphingomyelinase. The corresponding nucleic acid sequences are disclosed as SEQ. ID. NO. 3 and SEQ ID. NO. 4, respectively.

Parts of the nucleic acid sequences are identical with the EST sequences AA028477 and AA013912 (murine) and W32352 and AA056024 (human).

When he knows the amino acid and nucleic acid structure of human and murine neutral sphingomyelinase, one skilled in the art can easily detect the corresponding nucleic acids and proteins from other eukaryotes, considering the high homology between human and murine nSMases. To do this, he can either use cross-reacting antibodies for a purification by specific affinity chromatography, or he can synthesize oligonucleotide primers on the basis of the nucleic acid sequence and amplify the desired nucleic acids in a cDNA library of the eukaryote using polymerase chain reaction. The corresponding cDNA library can be obtained in a per se known manner by isolating mRNA from a tissue sample, followed by reverse transcription. From the nucleic acid sequence, the amino acid sequence can be derived by means of the genetic code. Alternatively, it is also possible to search for homologous sequences in EST (expressed sequence tags) data bases and combine them.

The nucleic acids according to the invention are suitable for the expression of eukaryotic neutral sphingomyelinase in prokaryotic or eukaryotic systems. In addition, they are also suitable for expression of nSMase in vivo in a gene therapy, or especially, in the form of fragments with complementary structures, they are also suitable as antisense nucleotides for reducing the expression of nSMase.

The nucleic acids according to the invention can be prepared by chemical synthesis or by amplification in genetically engineered organisms by methods per se known to those skilled in the art.

The invention also relates to eukaryotic neutral sphingomyelinase obtainable by the expression of the nucleic acids according to the invention.

The nSMase according to the invention can be prepared by expression in genetically engineered organisms. Eukaryotic expression systems are particularly suitable. Appropriate eukaryotic expression systems are known to those skilled in the art, for example, pRc/CMV (Stratagene). Purification from genetically engineered organisms offers an easy and direct access to the nSMase according to the invention, especially in the case of overexpression, and in addition allows for the isolation thereof in larger quantities.

The eukaryotic neutral sphingomyelinase is preferably a mammal, especially human or murine, neutral sphingomyelinase. The amino acid sequences of the human and murine neutral sphingomyelinases are represented as SEQ. ID. NOS. 1 and 2.

The molecular weights of human and murine sphingomyelinases are 47.6 and 47.5 kDa, respectively. In contrast to bacterial nSMases, the mammal nSMases according to the invention do not contain a signal sequence at the N terminus. From the hydrophobicity analysis, it can be considered that two neighboring hydrophobic membrane domains at the C terminus are separated by eight amino acids. Therefore, the proteins appear to be integral membrane proteins whose catalytically active domain is directed towards the cytosol while only a small proportion of the enzymes contacts the extracellular environment. This is in contrast to bacterial nSMases which are secreted, soluble proteins, but in agreement with previous studies on the properties of neutral sphingomyelinases of mammals. According to a Northern blot analysis, the 1.7 kb mRNA of murine nSMase is expressed in all tissues. In the kidneys, brain, liver, heart and lungs, the Northern blot shows a strong signal while expression in the spleen appears to be low. This measurement was not in agreement with the measured enzymatic activities of the corresponding tissues. This speaks in favor of a post-transcriptional regulation of nSMase.

The pH optimum of the neutral sphingomyelinase according to the invention is within a range of from 6.5 to 7.5, with a K_m value for C18 sphingomyelin

within a range of from 1.0 to 1.5 x 10^{-5} M. The activity is dependent on the presence of magnesium ions; the addition of EDTA results in an inhibition of SMase activity, which can be restored, however, by the addition of Mn^{2+} or Mg^{2+} ions. The addition of 0.3 to 0.5% Triton X-100 increases the enzymatic activity. The activity is not affected by a treatment with DTT or 2-mercaptoethanol whereas the addition of 20 mM glutathione led to inhibition. The activity of nSMase is not restricted to sphingomyelin; the structurally related phosphatidylcholine was also cleaved with about 3% activity.

Also claimed are variants of the eukaryotic neutral sphingomyelinase. The term "variants" encompasses both naturally occurring allelic variations of the eukaryotic neutral sphingomyelinase and proteins prepared by recombinant DNA technology (especially by in-vitro mutagenesis using chemically synthesized oligonucleotides) followed by expression which correspond to eukaryotic neutral sphingomyelinase in terms of biological and/or immunological activity. This may include the deletion, insertion or conservative substitution of amino acids. "Conservative substitution" means that an amino acid is substituted by another amino acid having similar physicochemical properties.

Thus, for example, the following amino acids are interchangeable: serine and alanine; alanine and glycine; methionine and serine; lysine and arginine; lysine and serine.

In particular, the term "variants" also includes N-terminally and/or C-terminally truncated proteins as well as acetylated, glycosylated, amidated and/or phosphorylated derivatives.

At least part of the activity of nSMase seems to reside in the C-terminal region since the fragment 1-282 of murine nSMase failed to exhibit an increase of sphingomyelinase activity when expressed in HEK293 cells. This invention also relates to C-terminal fragments of nSMase. Compounds in which nSMase or its variants are coupled with other molecules, such as dyes, radionuclides or affinity components, are also variants according to the invention.

Also claimed are nucleic acids coding for eukaryotic neutral sphingomyelinase or being complementary to such nucleic acids. The nucleic acids may be, for example, DNA, RNA, PNA or nuclease-resistant analogues thereof. In particular, nuclease-resistant analogues include those compounds which have the phosphodiester linkage modified by hydrolysis-stable compounds, such as phosphothioates, methylphosphonates or the like.

Especially short fragments of the nucleic acids are suitable as antisense nucleotides. For reasons of specificity, they should preferably contain more than 6, more preferably more than 8 and most preferably more than 12 nucleotides. For reasons of diffusion and costs, they usually have a length of less than 30 nucleotides, preferably 24 or less, and more preferably 18 or less nucleotides.

The invention also relates to derivatives of nucleic acids which are coupled to other molecules for diagnostic or therapeutic purposes, for example, to fluorescent dyes, radioactive labels or affinity components, and fragments of the nucleic acids according to the invention, and the nucleic acids complementary to these nucleic acids, and variants of the nucleic acids.

"Fragments" as used herein means nucleic acids truncated at the 5' or 3' or at both ends. The term "variants" means that these nucleic acids will hybridize with the nucleic acid according to the invention or with nucleic acids complementary thereto under stringent conditions. The term "stringent conditions" means that the hybridization is performed under conditions in which the temperature is even lower by up to 10 °C than the temperature (conditions being otherwise identical) just low enough for exactly complementary nucleic acids to anneal. For example, if an exactly complementary nucleic acid will anneal down to a temperature of about 55 °C under given conditions, then stringent conditions are temperatures of equal to or higher than 45 °C. Preferably, the temperature range for stringent conditions is within 5 °C, more preferably within 3 °C.

Further, the invention relates to antibodies directed against the nSMase according to the invention or the nucleic acids according to the invention.

These substances are suitable, in particular, for use in diagnostics, in immuno-assays per se known to those skilled in the art, for histological studies and as medicaments for the treatment of conditions associated with an overexpression of nSMase. Such antibodies according to the invention can be obtained by methods per se known to those skilled in the art through immunization with nSMase, nucleic acids according to the invention or peptide and nucleic acid fragments in the presence of adjuvants.

Further, the invention relates to cell lines which overexpress the nSMase according to the invention. Such cell lines can be obtained by transfection with vectors containing the nucleic acids according to the invention coding for nSMase. In the case of eukaryotic cell lines, for example, transfection may be effected by electroporation. Preferably, the cell lines are stably transfected.

In this connection, "overexpression" means that the cell line has a higher activity of nSMase than cell lines which have not been transfected with the nucleic acids according to the invention. For example, suitable eukaryotic cell lines include the cell lines U937, HEK 293 or Jurkat.

In experiments, the cell lines exhibited a specific nSMase activity of between 0.3 and 10 µmol/mg of protein/hour.

Figure 3 shows the Northern and Western blot analysis of nSMase expression in transfected cell lines. Portion A shows the result of a RT PCR of the whole cell RNA with primers hybridizing with human and murine nSMase cDNAs. Portion B shows the T PCR of the whole RNA with primers hybridizing with human β-actin cDNA as a control. Portion C shows the Western blot of the plasma membrane protein extract of different HEK 293 cell lines after SDS polyacrylamide gel electrophoresis and hybridization with polyclonal anti-nSMase antibodies.

The addition of 0.5 mM arachidonic acid resulted in a threefold increase of nSMase activity in the overexpressing HEK cells.

The invention further relates to a transgenic mammal which exhibits an overexpression (gain of function) or a genetic deficiency or defect (loss of function) for the nSMase according to the invention. The mammal is preferably a rodent, especially a mouse. Such transgenic mammals can be obtained by methods per se known to those skilled in the art and are especially suitable for elucidating the function of neutral sphingomyelinase. For transgenic mammals, defined gene constructs are injected into the pronucleus of a fertilized egg cell by DNA microinjection to achieve the expression of an additional gene. By selectively changing a gene in the genome of ES cells which are subsequently injected in blastocysts, the function of a gene is switched off.

The strategy and constructs for generating the mouse mutants are shown in Figures 4 and 5.

The transgenic animals are preferably animals in which the gene can be switched on and off temporally and in a tissue-specific way by external induction. Such transgenic mammals are especially suitable for elucidating the metabolic and signal transduction pathways related to the nSMase according to the invention; this in turn enables diagnostic or therapeutic applications. In particular, the transgenic mammals are suitable for the screening of pharmaceutically active substances.

The eukaryotic neutral sphingomyelinase according to the invention, the nucleic acids according to the invention and the antibodies according to the invention can be contained in medicaments and diagnostic agents, optionally together with further auxiliary agents. Such medicaments and diagnostic agents are suitable for the diagnosis and treatment of diseases based on over- or underexpression and/or an increased or reduced activity of eukaryotic neutral sphingomyelinase and/or disorders of cell proliferation, cell differentiation and/or apoptosis.

In particular, these are diseases in which inflammation processes, cell growth disorders and metabolic disorders are involved. For example, they may be cancers or disorders of cholesterol homeostasis (atherosclerosis).

A pharmaceutical screening method according to the invention relies on a change of the expression or activity of the nSMase according to the invention in nSMase-overexpressing cell lines upon the addition of at least one potential pharmaceutically active substance. Thus, the cell lines are suitable, in particular, for developing and testing pharmaceutical leading structures.

The invention will be further illustrated by the following Examples.

Example 1

Cloning of the nucleic acid

The inventive nucleic acids coding for neutral sphingomyelinase were cloned into the NotI restriction sites of the cloning site of the eukaryotic expression vector pRc/CMV (Stratagene). The sequences of the resulting DNAs were obtained by sequencing using a Perkin-Elmer DNA sequencer 377A.

Example 2

Cloning of the RNA

The whole RNA was isolated from different organs of eight three-week-old CD1 mice according to known methods, and poly(A^+) RNA was isolated by affinity purification on oligo(dT) cellulose (Boehringer Mannheim, Germany) according to standard methods.

Example 3

Overexpressing cell lines

U937 cells were grown in PRMI 1640 medium with 10% fetal calf serum, 1 µg/ml penicillin/streptomycin and 0.03% glutamine at 37 °C and 5% CO_2. By electroporation with a Gene Pulser (Bio-Rad), 5 x 10^6 cells were transfected with 1 µg of linearized plasmid DNA coding for the nSMase according

to the invention. The selection of stable clones was effected by using 1 mg/ml geneticin (G418, Life Technologies, Gaithersburg, MD).

The nSMase purified from the cell lines exhibited a specific activity of between 0.3 and 10 µmol/mg of protein/hour. Its pH optimum was at 6.5 and 7.5. The K_M value for C18 sphingomyelin was from 1.0 to 1.5 x 10^{-5} M. The activity was dependent on the presence of magnesium ions; the addition of EDTA inhibited the activity.

Example 4

Measurement of nSMase activity

The enzymatic activity was examined in cells and murine tissues. The cells were washed twice with ice-cold PBS and sedimented at 1,000 x g. The pellet was resuspended in lysis buffer, and the cells were disrupted by repeated cycles of freezing and thawing. After centrifugation at 2,500 x g for 2 min, extraction with lysis buffer containing 0.2% Triton X-100 was performed, followed by centrifugation at 100,000 x g for 15 min.

Tissue from three-week-old mice was homogenized in cold lysis buffer. The quantity of protein or homogenized tissue to be examined was incubated with 10 nM (80,000 dpm) [N-$^{14}CH_3$]sphingomyelin for 30 min at 37 °C in a total volume of 200 µl. Then, 100 µl of water was added, and unreacted substrate was removed by extraction with chloroform-methanol (2:1, v/v). The radioactivity of the aqueous phase containing the enzymatically released phosphocholine was measured in a scintillation counter.

Example 5

Polyclonal antibodies

Rabbits were immunized with the synthetic peptide CDPHSDKPFSDHE (corresponding to amino acids 261 through 273 of murine nSMase), coupled to keyhole limpet hemocyanin. The polyclonal antibody serum was purified by

chromatography on hydroxyapatite and affinity chromatography on a column having the above mentioned synthetic peptide bound thereto.

CLAIMS:

(amended August 24, 1999)

1. A eukaryotic neutral sphingomyelinase having the sequence according to SEQ. ID. NO. 1 or SEQ. ID. NO. 2 and variants of said eukaryotic neutral sphingomyelinase of SEQ. ID. NO. 1 or SEQ. ID. NO. 2 which correspond to eukaryotic neutral sphingomyelinase in terms of biological and/or immunological activity.

2. A eukaryotic neutral sphingomyelinase, characterized by being a C-terminally or N-terminally truncated variant.

3. A nucleic acid coding for the eukaryotic neutral sphingomyelinase according to claim 1 or 2.

4. The nucleic acid according to claim 3 having the sequence according to SEQ. ID. NO. 3 or SEQ. ID. NO. 4.

5. The nucleic acid according to at least one of claims 3 to 4, characterized by being DNA, RNA, PNA or nuclease-resistant analogues thereof, mRNA, cDNA or genomic DNA.

6. The nucleic acids according to claim 5, characterized by being the gene for eukaryotic neutral sphingomyelinase which contains non-coding regions (introns) in addition to coding regions (exons), especially a gene having the sequence according to SEQ. ID. NO. 5 or SEQ. ID. NO. 6.

7. A nucleic acid, characterized by being complementary to the nucleic acid according to at least one of claims 3 to 6.

8. The nucleic acid according to at least one of claims 3 to 7, characterized by being derivatives, fragments with more than six nucleotides or variants of such nucleic acids.

9. Antibodies, characterized by being directed against the eukaryotic neutral sphingomyelinase according to any of claims 1 or 2 or a nucleic acid according to at least one of claims 3 to 8.

10. A cell line, characterized by overexpressing the neutral sphingomyelinase according to claim 1 or 2.

11. The cell line according to claim 10, characterized by being a cell line which expresses eukaryotic neutral sphingomyelinase and is based on the cell lines U937, HEK 293 or Jurkat.

12. A transgenic mammal exhibiting overexpression (gain of function) or a genetic deficiency or defect (loss of function) for eukaryotic neutral sphingomyelinase according to claim 1 or 2.

13. The transgenic mammal according to claim 12, characterized by being a rodent.

14. A medicament containing the eukaryotic neutral sphingomyelinase according to any of claims 1 or 2, a nucleic acid according to at least one of claims 3 to 8, and/or an antibody according to claim 9, together with further auxiliary agents.

15. A diagnostic agent containing the eukaryotic neutral sphingomyelinase according to any of claims 1 or 2, a nucleic acid according to at least one of claims 3 to 8, and/or an antibody according to claim 9, together with further auxiliary agents.

16. Use of the medicaments according to claim 14 or the diagnostic agents according to claim 15 for the diagnosis and treatment of diseases based on over- or underexpression and/or an increased or reduced activity of eukaryotic neutral sphingomyelinase and/or disorders of cell proliferation, cell differentiation and/or apoptosis.

17. The use according to claim 16, characterized in that said diseases are inflammation processes, cell growth disorders, cancers and/or meta-

bolic disorders, such as disorders of cholesterol homeostasis (atherosclerosis).

18. A method for the screening of active substances, characterized in that a change in expression or activity of the eukaryotic neutral sphingomyelinase is measured in cell lines according to claim 10 upon the addition of at least one potential pharmaceutically active substance.

19. Use of the cell line according to claim 10 for developing and testing pharmaceutical leading structures.

20. A process for the preparation of the eukaryotic neutral sphingomyelinase according to any of claims 1 or 2 by chemical peptide synthesis or by expression in genetically engineered organisms, especially in eukaryotic expression systems.

21. A process for the preparation of a nucleic acid according to at least one of claims 3 to 8 by chemical synthesis or by amplification in genetically engineered organisms.

Abstract

The present invention relates to eukaryotic neutral sphingomyelinase (nSMase) and its application.

human neutral Sphingomyelinase (NSM) Gene Sequence

```
      ACCGCGGCCGTCGCTGGAGAGTTCGAGCCGCCTAGCGCCCCTGGAGCTCCCCAACCATGA
    1 ---------+---------+---------+---------+---------+---------+   60
      TGGCGCCGGCAGCGACCTCTCAAGCTCGGCGGATCGCGGGGACCTCGAGGGGTTGGTACT

                                                                      E I
      AGCCCAACTTCTCCCTGCGACTGCGGATCTTCAACCTCAACTGCTGGTGAGTGCGTCTGC
   61 ---------+---------+---------+---------+---------+---------+  120
      TCGGGTTGAAGAGGGACGCTGACGCCTAGAAGTTGGAGTTGACGACCACTCACGCAGACG

      GGAGTGCGGTCTGGGGGCCACCTTCCGTTCGCACCCATGCAGCCTTCCTCCCCCTATCCC
  121 ---------+---------+---------+---------+---------+---------+  180
      CCTCACGCCAGACCCCCGGTGGAAGGCAAGCGTGGGTACGTCGGAAGGAGGGGGATAGGG

      GCCCCACGATCTCAGGGTGTAGGGAAAACCCGAACCTCCAAAGTCCACATCTGGCCCCAG
  181 ---------+---------+---------+---------+---------+---------+  240
      CGGGGTGCTAGAGTCCCACATCCCTTTTGGGCTTGGAGGTTTCAGGTGTAGACCGGGGTC

      CGCCGGTGGTCCCAGCAGTCGCCTCCCCTGCCCCGCTCTTCCCTTCCTTAGGGGCATTCC
  241 ---------+---------+---------+---------+---------+---------+  300
      GCGGCCACCAGGGTCGTCAGCGGAGGGGACGGGGCGAGAAGGGAAGGAATCCCCGTAAGG

      GTACTTGAGCAAGCACCGGGCCGACCGCATGAGGCGCCTGGGAGACTTTCTGAACCAGGA
  301 ---------+---------+---------+---------+---------+---------+  360
      CATGAACTCGTTCGTGGCCCGGCTGGCGTACTCCGCGGACCCTCTGAAAGACTTGGTCCT

                                                                      E II
      GAGCTTCGACCTGGCTTTGCTGGAGGAGGTGAGATTGTGCAGCACGGTGCGGAACCCAGG
  361 ---------+---------+---------+---------+---------+---------+  420
      CTCGAAGCTGGACCGAAACGACCTCCTCCACTCTAACACGTCGTGCCACGCCTTGGGTCC

      CTGGGAGGAGGGACAGACCGTCCCACTGGGGAAAGACCAAGCAGGCATCCTCACCGCTTC
  421 ---------+---------+---------+---------+---------+---------+  480
      GACCCTCCTCCCTGTCTGGCAGGGTGACCCCTTTCTGGTTCGTCCGTAGGAGTGGCGAAG

      CCTCAGGTGTGGAGTGAGCAGGACTTCCAGTACCTGAGACAGAAGCTGTCACCTACCTAC
  481 ---------+---------+---------+---------+---------+---------+  540
      GGAGTCCACACCTCACTCGTCCTGAAGGTCATGGACTCTGTCTTCGACAGTGGATGGATG

                                                                      E III
      CCAGCTGCACACCACTTCCGGAGGTGAGAAGCCCACTGGCCTGAAGCCTGTTGTCATCCC
  541 ---------+---------+---------+---------+---------+---------+  600
      GGTCGACGTGTGGTGAAGGCCTCCACTCTTCGGGTGACCGGACTTCGGACAACAGTAGGG

      AGGAGGCTCTTGGCCCTGCCAGCCCTTCCCTATCCTGCCTGCACTCTCCAGTCTCCTCCA
  601 ---------+---------+---------+---------+---------+---------+  660
      TCCTCCGAGAACCGGGACGGTCGGGAAGGGATAGGACGGACGTGAGAGGTCAGAGGAGGT

      GCCTCCTCTCCCTCTGGATGTGAGAGAAGGAGAAGGGTGAACCAAGAAGGTCCTATGACT
  661 ---------+---------+---------+---------+---------+---------+  720
      CGGAGGAGAGGGAGACCTACACTCTCTTCCTCTTCCCACTTGGTTCTTCCAGGATACTGA

      TCAGCCCATTTCAGCTTTGTTTTCTGGCTGCCCTATACTCCTCCAAAGGCCGTCGCCTTG
  721 ---------+---------+---------+---------+---------+---------+  780
      AGTCGGGTAAAGTCGAAACAAAAGACCGACGGGATATGAGGAGGTTTCCGGCAGCGGAAC

      GTTCTAGGGCTAGTCCCAGCAGTAGAAAAAGAAAAAAATAGCTGATCAGAGCTGGAAGAC
  781 ---------+---------+---------+---------+---------+---------+  840
      CAAGATCCCGATCAGGGTCGTCATCTTTTTCTTTTTTTATCGACTAGTCTCGACCTTCTG

      AAGGGAGGGGAAGAAGGCTGGGTGTCTCTCCCTGTTTTTCTGGTTATTAAGCAGGGCTTG
  841 ---------+---------+---------+---------+---------+---------+  900
      TTCCCTCCCCTTCTTCCGACCCACAGAGAGGGACAAAAAGACCAATAATTCGTCCCGAAC
```

Figure 1-1

```
                CTCTCCCTCCTTCTCCCCCACATCCTAGCATGAGCCAATGATTCCCTTAGGGCTCTGAGG
1861            ---------+---------+---------+---------+---------+---------+
                GAGAGGGAGGAAGAGGGGGTGTAGGATCGTACTCGGTTACTAAGGGAATCCCGAGACTCC  1920

                                                                          E VIII
                AAGGCAACACAATGGTACCCAAGAACTGNTACGTCAGCCAGCAGGAGCTGAAGCCATTTC
1921            ---------+---------+---------+---------+---------+---------+
                TTCCGTTGTGTTACCATGGGTTCTTGACNATGCAGTCGGTCGTCCTCGACTTCGGTAAAG  1980

                CCTTTGGTGTCCGCATTGACTACGTGCTTTACAAGGTCAGGCTCCTCCCTTCAACATGCT
1981            ---------+---------+---------+---------+---------+---------+
                GGAAACCACAGGCGTAACTGATGCACGAAATGTTCCAGTCCGAGGAGGGAAGTTGTACGA  2040

                TTCATATGCTGTGTCTCTTTGTCTACTAACCTGTGTAGATCCTTTGCTCAGNTAGTCTAG
2041            ---------+---------+---------+---------+---------+---------+
                AAGTATACGACACAGAGAAACAGATGATTGGACACATCTAGGAAACGAGTCNATCAGATC  2100

                TCTTGGACCACTGATGGGTGGAAAGTGGGGTAGCCGGGAGCTGGTTCTCTGGGAAGAGGC
2101            ---------+---------+---------+---------+---------+---------+
                AGAACCTGGTGACTACCCACCTTTCACCCCATCGGCCCTCGACCAAGAGACCCTTCTCCG  2160

                CCTCATATATAAGCTTCTCTNTGGCCCTTACTTTTCCTAGGCAGTTTCTGGGTTTTACAT
2161            ---------+---------+---------+---------+---------+---------+
                GGAGTATATATTCGAAGAGANACCGGGAATGAAAAGGATCCGTCAAAGACCCAAAATGTA  2220

                CTCCTGTAAGAGTTTTGAAACCACTACAGGCTTTGACCCTNACAGGGGCACCCCCCTCTC
2221            ---------+---------+---------+---------+---------+---------+
                GAGGACATTCTCAAAACTTTGGTGATGTCCGAAACTGGGANTGTCCCCGTGGGGGGAGAG  2280

                                                                          E IX
                TTGATCATGAAGCCCTGATGGCTACTCTGTTTGTGAGGCACAGCCCCCCACAGCAGAACC
2281            ---------+----- ----+---------+---------+---------+---------+
                AACTAGTACTTCGGGACTACCGATGAGACAAACACTCCGTGTCGGGGGGTGTCGTCTTGG  2340

                CCAGCTCTACCCACGGTGAGTCACCCCCACCCTTTCCTTGGCCCTTGCCCCGCTTGAAGC
2341            ---------+---------+---------+---------+---------+---------+
                GGTCGAGATGGGTGCCACTCAGTGGGGGTGGGAAAGGAACCGGGAACGGGGCGAACTTCG  2400

                AGCCCTTCCACTCTTGACTCTCTCCTGCCCCACTGCCCTGCTCTGTTGTAGGACCAGCAG
2401            ---------+---------+---------+---------+---------+---------+
                TCGGGAAGGTGAGAACTGAGAGAGGACGGGGTGACGGGACGAGACAACATCCTGGTCGTC  2460

                AGAGGTCGCCGTTGATGTGTGTGCTAAAGGAGGCCTGGACGGAGCTGGGTCTGGGCATGG
2461            ---------+---------+---------+---------+---------+---------+
                TCTCCAGCGGCAACTACACACACGATTTCCTCCGGACCTGCCTCGACCCAGACCCGTACC  2520

                CTCAGGCTCGCTGGTGGGCCACCTTCGCTAGCTATGTGATTGGCCTGGGGCTGCTTCTCC
2521            ---------+---------+---------+---------+---------+---------+
                GAGTCCGAGCGACCACCCGGTGGAAGCGATCGATACACTAACCGGACCCCGACGAAGAGG  2580

                                                                          E X
                TGGCACTGCTGTGTGTCCTGGCGGCTGGAGGAGGGGCCGGGGAAGCTGCCATACTGCTCT
2581            ---------+---------+---------+---------+---------+---------+
                ACCGTGACGACACACAGGACCGCCGACCTCCTCCCCGGCCCCTTCGACGGTATGACGAGA  2640

                GGACCCCCAGTGTAGGGCTGGTGCTGTGGGCAGGTGCATTCTACCTCTTCCACGTACAGG
2641            ---------+---------+---------+---------+---------+---------+
                CCTGGGGGTCACATCCCGACCACGACACCCGTCCACGTAAGATGGAGAAGGTGCATGTCC  2700

                AGGTCAATGGCTTATATAGGGCCCAGGCTGAGCTCCAGCATGTGCTAGGAAGGGCAAGGG
2701            ---------+---------+---------+---------+---------+---------+
                TCCAGTTACCGAATATATCCCGGGTCCGACTCGAGGTCGTACACGATCCTTCCCGTTCCC  2760

                AGGCCCAGGATCTGGGCCCAGAGCCTCAGCCAGCCCTACTCCTGGGGCAGCAGGAGGGGG
2761            ---------+---------+---------+---------+---------+---------+
                TCCGGGTCCTAGACCCGGGTCTCGGAGTCGGTCGGGATGAGGACCCCGTCGTCCTCCCCC  2820

                 ACAGAACTAAAGAACAATAAAGCTTGGCCCAA
```

Figure 1-2

```
2821 ---------+---------+---------+-- 2852
     TGTCTTGATTTCTTGTTATTTCGAACCGGGTT
```


Figure 1-3

Mouse Neutral Sphingomyelinase (nSMase) gene sequence

```
     TNGANNCTGTTAGCTCCAGNCCGGTNGGTCGCCGTNCTAGNCNNATCTNTATAGCTCTTC
   1 ---------+---------+---------+---------+---------+---------+ 60
     ANCTNNGACAATCGAGGTCNGGCCANCCAGCGGCANGATCNGNNTAGANATATCGAGAAG

     GTTGCGAGCNCAATTNNNTCTCAATAAANGGATNCANCCCTATGACAGAACGTGGACCCC
  61 ---------+---------+---------+---------+---------+---------+ 120
     CAACGCTCGNGTTAANNNAGAGTTATTTNCCTANGTNGGGATACTGTCTTGCACCTGGGG

     CGCCCGCCANCNCANGNGANACCGCGGCATGGGNCTGAGGTGCNCANGGTGTCTGGGGCG
 121 ---------+---------+---------+---------+---------+---------+ 180
     GCGGGCGGTNGNGTNCNCTNTGGCGCCGTACCCNGACTCCACGNGTNCCACAGACCCCGC

     AGGGGTTACCTCAGCGATGGTCTTTGACACCTGAAAGCTGGAGCTTTTGAANAGCCCCAN
 181 ---------+---------+---------+---------+---------+---------+ 240
     TCCCCAATGGAGTCGCTACCAGAAACTGTGGACTTTCGACCTCGAAAACTTNTCGGGGTN

     CACCTTCAGCTTCAGGGGCGGCTCNGGCGGCAACCGCACGTGANATGCTGGGGGCTTCGA
 241 ---------+---------+---------+---------+---------+---------+ 300
     GTGGAAGTCGAAGTCCCCGCCGAGNCCGCCGTTGGCGTGCACTNTACGACCCCCGAAGCT

     CTTGGGCCGGCACGGNTGCTGGGTGGCCATGGAANNNACAGNACAGAGCCCGGNACACAA
 301 ---------+---------+---------+---------+---------+---------+ 360
     GAACCCGGCCGTGCCNACGACCCACCGGTACCTTNNNTGTCNTGTCTCGGGCCNTGTGTT

     ATANTGCGAGTCGCCANGGNAACCGCGTGGCTCCTCCCCGAACGCCCNCAAGGGGCGGGA
 361 ---------+---------+---------+---------+---------+---------+ 420
     TATNACGCTCAGCGGTNCCNTTGGCGCACCGAGGAGGGGCTTGCGGGNGTTCCCCGCCCT

     CCTGAGTGAGTTCNTGGGCGGGGCCTCNCATCAACTTCAAGCCTGTTGCTGGTGGAAGCC
 421 ---------+---------+---------+---------+---------+---------+ 480
     GGACTCACTCAAGNACCCGCCCCGGAGNGTAGTTGAAGTTCGGACAACGACCACCTTCGG

     GAGCCGGGAACAAGGGAGGAACCTGTAGGCCGCGGTGCGGATAACCCACCGAAGGACCTA
EI 481 ---------+---------+---------+---------+---------+---------+ 540
     CTCGGCCCTTGTTCCCTCCTTGGACATCCGGCGCCACGCCTATTGGGTGGCTTCCTGGAT

     AGAATCTGGAACAGTCCACCCGAGATTCCTTCCAGGACTGCCGGCGGACTCTCGCATTCA
 541 ---------+---------+---------+---------+---------+---------+ 600
     TCTTAGACCTTGTCAGGTGGGCTCTAAGGAAGGTCCTGACGGCCGCCTGAGAGCGTAAGT

     GCCCGGGATTTGCAGCCGACCTTCTTTCCGGGTGGAATGACGGCCTTTGTCCCAGTAACG
 601 ---------+---------+---------+---------+---------+---------+ 660
     CGGGCCCTAAACGTCGGCTGGAAGAAAGGCCCACCTTACTGCCGGAAACAGGGTCATTGC

     CAGGAGTCNNCCCCACCCCCAACCAGCTCGCGTTCCTGGGTCGGGGCAGCGCAGGATAGG
 661 ---------+---------+---------+---------+---------+---------+ 720
     GTCCTCAGNNGGGGTGGGGGTTGGTCGAGCGCAAGGACCCAGCCCCGTCGCGTCCTATCC

                                                          Start
     GCAATAAGCCTGTGCGCGCAATCCGCCTCGCCGCCCTTGCTCCGAAGCACTCCAGCCATG
 721 ---------+---------+---------+---------+---------+---------+ 780
     CGTTATTCGGACACGCGCGTTAGGCGGAGCGGCGGGAACGAGGCTTCGTGAGGTCGGTAC

     AAGCTCAACTTTTCTCTACGGCTGAGAGTTTTCAATCTCAACTGCTGgtaagtaagtgct
 781 ---------+---------+---------+---------+---------+---------+ 840
     TTCGAGTTGAAAAGAGATGCCGACTCTCAAAAGTTAGAGTTGACGACcattcattcacga
```

Figure 2-1

```
     cccaggcgtgggCTGCAGCCTCGGAGCCACCTTCCAGTCCCCTCTCGCACATGCCTAGGA
 841 ---------+---------+---------+---------+---------+---------+ 900
     gggtccgcacccGACGTCGGAGCCTCGGTGGAAGGTCAGGGGAGAGCGTGTACGGATCCT

     AGGAAGCAGGTCTTCTTCAGCCGAGCTAGACCCTGTCCTTCCCGAACCACCAAAGTCCAC
 901 ---------+---------+---------+---------+---------+---------+ 960
     TCCTTCGTCCAGAAGAAGTCGGCTCGATCTGGGACAGGAAGGGCTTGGTGGTTTCAGGTG

     ATCGCCTAAAGACCAGAGCTTGGGTGGTTGCAGCAATCACCAAAGTCCCTATCATCCAAA
 961 ---------+---------+---------+---------+---------+---------+ 1020
     TAGCGGATTTCTGGTCTCGAACCCACCAACGTCGTTAGTGGTTTCAGGGATAGTAGGTTT

     GCTGAGGTGATGACAGCAGTAATCGTCCCAAACCTGGCCCATGTCTTTCCTTTTAAATGA
1021 ---------+---------+---------+---------+---------+---------+ 1080
     CGACTCCACTACTGTCGTCATTAGCAGGGTTTGGACCGGGTACAGAAAGGAAAATTTACT

     TTTACTTTTATTTTATGTACATTTGGTGTTTTGCCTGTATGTATGTCTGTGTGAAGGTGC
1081 ---------+---------+---------+---------+---------+---------+ 1140
     AAATGAAAATAAAATACATGTAAACCACAAAACGGACATACATACAGACACACTTCCACG

     CAGATTCTCTGGAACTGGAGTTACAGACAGTTGTAAGCTGTCATGTGCTTGCTGGAAATT
1141 ---------+---------+---------+---------+---------+---------+ 1200
     GTCTAAGAGACCTTGACCTCAATGTCTGTCAACATTCGACAGTACACGAACGACCTTTAA

     GAACTGCTGACCCATCTCTTCTGCCCCCTGCGTCCTCCACCCCTTTTAGGGACATCCCCT
1201 ---------+---------+---------+---------+---------+---------+ 1260
     CTTGACGACTGGGTAGAGAAGACGGGGGACGCAGGAGGTGGGGAAAATCCCTGTAGGGGA

     ACCTGAGCAAACATAGGGCGGACCGCATGAAGCGCTTGGGAGACTTTCTGAACTTGGAAA
1261 ---------+---------+---------+---------+---------+---------+ 1320
     TGGACTCGTTTGTATCCCGCCTGGCGTACTTCGCGAACCCTCTGAAAGACTTGAACCTTT
```

E II

```
     ACTTTGATCTGGCTCTCCTGGAGGAGGTGAGGTTGTAGGGCAGGCTAGGTTGGAGGAGGG
1321 ---------+---------+---------+---------+---------+---------+ 1380
     TGAAACTAGACCGAGAGGACCTCCTCCACTCCAACATCCCGTCCGATCCAACCTCCTCCC

     CAGCAGGCGGCAGGCGGCGGCAGGAAAACTTGTTCTGTCTTGGGATGAAATCCCAAGCAA
1381 ---------+---------+---------+---------+---------+---------+ 1440
     GTCGTCCGCCGTCCGCCGCCGTCCTTTTGAACAAGACAGAACCCTACTTTAGGGTTCGTT

     GTATCCTCACCTTCTTCCTCCAGGTGTGGAGTGAGCAGGACTTCCCAGTACCTAAGGCAA
1441 ---------+---------+---------+---------+---------+---------+ 1500
     CATAGGAGTGGAAGAAGGAGGTCCACACCTCACTCGTCCTGAAGGGTCATGGATTCCGTT
```

E III

```
     AGGCTATCGCTCACCTATCCAGATGCACACTACTTCAGAAGGTGAAAAGCCTGTGTTCTC
1501 ---------+---------+---------+---------+---------+---------+ 1560
     TCCGATAGCGAGTGGATAGGTCTACGTGTGATGAAGTCTTCCACTTTTCGGACACAAGAG

     AGCCTGTTCTCAGACGAGGAAGCTCTCCAACATTCTTGCTTGCACCCTCGATCTTCTTCC
1561 ---------+---------+---------+---------+---------+---------+ 1620
     TCGGACAAGAGTCTGCTCCTTCGAGAGGTTGTAAGAACGAACGTGGGAGCTAGAAGAAGG

     TCTGGGTGTGAGAAGAGCAGGCCGTCACCCTCATCTTGCAAGGGCTGCTGTCTTAGGCTT
1621 ---------+---------+---------+---------+---------+---------+ 1680
     AGACCCACACTCTTCTCGTCCGGCAGTGGGAGTAGAACGTTCCCGACGACAGAATCCGAA

     TGTTCTGGGGTTGATCTTAGCAGTAGAGCTGGGAGACCGCGGAGGGGAAGAGGGCTGGCT
1681 ---------+---------+---------+---------+---------+---------+ 1740
     ACAAGACCCCAACTAGAATCGTCATCTCGACCCTCTGGCGCCTCCCCTTCTCCCGACCGA
```

Figure 2-2

```
                GGGTACTCCCCTCCTTGCTCTTCTGGTTATTAAGCAAGAGTTGGTTTTCAGCGGGATGAT
      1741      ---------+---------+---------+---------+---------+---------+ 1800
                CCCATGAGGGGAGGAACGAGAAGACCAATAATTCGTTCTCAACCAAAAGTCGCCCTACTA
E IV

                AGGCAGTGGCCTCTGTGTGTTCTCCAAACACCCAATCCAGGAAATCTTCCAGCATGTCTA
      1801      ---------+---------+---------+---------+---------+---------+ 1860
                TCCGTCACCGGAGACACACAAGAGGTTTGTGGGTTAGGTCCTTTAGAAGGTCGTACAGAT

                CAGTCTGAATGGTTACCCCTACATGGTAAGGATCTCTTCCCTATCCTTGCTAACACAGAC
      1861      ---------+---------+---------+---------+---------+---------+ 1920
                GTCAGACTTACCAATGGGGATGTACCATTCCTAGAGAAGGGATAGGAACGATTGTGTCTG

                TGGACGCAGCCTTCCTGGGGCCTTGGCAGGAGGGTGTCAGTACCCTGAGTTTTTGTCTTC
      1921      ---------+---------+---------+---------+---------+---------+ 1980
                ACCTGCGTCGGAAGGACCCCGGAACCGTCCTCCCACAGTCATGGGACTCAAAAACAGAAG

                TCTTGCCTGCAGTTCCATCATGGAGACTGGTTCTGTGGGAAGTCTGTGGGGCTGCTGGTG
      1981      ---------+---------+---------+---------+---------+---------+ 2040
                AGAACGGACGTCAAGGTAGTACCTCTGACCAAGACACCCTTCAGACACCCCGACGACCAC
E V

                CTCCGTCTAAGTGGACTGGTGCTCAATGCCTACGTGACTCATGTGAGTGGGGCTAGCCAG
      2041      ---------+---------+---------+---------+---------+---------+ 2100
                GAGGCAGATTCACCTGACCACGAGTTACGGATGCACTGAGTACACTCACCCCGATCGGTC

                GCTTAGGCAGTGGGTCAAGCAGCCCAATGCTATGGTGGAGAAGAGACGCCACTAGTTAGT
      2101      ---------+---------+---------+---------+---------+---------+ 2160
                CGAATCCGTCACCCAGTTCGTCGGGTTACGATACCACCTCTTCTCTGCGGTGATCAATCA

                TCTGCTGCCTGGGGATAAGGCATGGGATCAGAAGCTAGCATTGGGCAAGGTTCACCCATT
      2161      ---------+---------+---------+---------+---------+---------+ 2220
                AGACGACGGACCCCTATTCCGTACCCTAGTCTTCGATCGTAACCCGTTCCAAGTGGGTAA

                CCCTGTCACACTCTGCCATGTGACAGATGACAAGCTTGATTCAGACAGCCTTCTCTTTGA
      2221      ---------+---------+---------+---------+---------+---------+ 2280
                GGGACAGTGTGAGACGGTACACTGTCTACTGTTCGAACTAAGTCTGTCGGAAGAGAAACT

                TTTCACCTATTCCACTTTAGCTACATGCTGAGTACAGCCGACAGAAGGACATCTACTTTG
      2281      ---------+---------+---------+---------+---------+---------+ 2340
                AAAGTGGATAAGGTGAAATCGATGTACGACTCATGTCGGCTGTCTTCCTGTAGATGAAAC
E VI

                CACACCGTGTGGCCCAAGCTTGGGAACTGGCCCAGTTCATCCAGTGTGTGAGCCTGGGCT
      2341      ---------+---------+---------+---------+---------+---------+ 2400
                GTGTGGCACACCGGGTTCGAACCCTTGACCGGGTCAAGTAGGTCACACACTCGGACCCGA

                TGATGGGGGCTGTGGGGTGGGGACGGGGTTGAGGGATGNGNAANTTATCCTTGAAGAGGG
      2401      ---------+---------+---------+---------+---------+---------+ 2460
                ACTACCCCCGACACCCCACCCCTGCCCCAACTCCCTACNCNTTNAATAGGAACTTCTCCC

                CACATAATAAGGGAAGAATTTCCTCCTTGCCGCTCTTCCCCCAACTCAGCCACACATCCA
      2461      ---------+---------+---------+---------+---------+---------+ 2520
                GTGTATTATTCCCTTCTTAAAGGAGGAACGGCGAGAAGGGGGTTGAGTCGGTGTGTAGGT
E VII

                AGAATGCAGATGTGGTTCTATTGTGTGGAGACCTCAATATGCACCCCAAAGACCTGGGCT
      2521      ---------+---------+---------+---------+---------+---------+ 2580
                TCTTACGTCTACACCAAGATAACACACCTCTGGAGTTATACGTGGGGTTTCTGGACCCGA
```

Figure 2-3

```
                GCTGCCTGCTGAAAGAGTGGACAGGGCTCCATGATGCTTTCGTTGAGACTGAGGACTTTA
2581  ---------+---------+---------+---------+---------+---------+---------+ 2640
                CGACGGACGACTTTCTCACCTGTCCCGAGGTACTACGAAAGCAACTCTGACTCCTGAAAT

                AGGTGAGAGACTGTTTCCCACCAACTCCACACTTGTTCCAGTCTTCCTGTCTCTTAGCAT
2641  ---------+---------+---------+---------+---------+---------+---------+ 2700
                TCCACTCTCTGACAAAGGGTGGTTGAGGTGTGAACAAGGTCAGAAGGACAGAGAATCGTA

                CCTAGCCACCTGTTTCCCTAGGGCTCTGATGATGGCTGTACCATGGTACCCAAGAACTGC
2701  ---------+---------+---------+---------+---------+---------+---------+ 2760
                GGATCGGTGGACAAAGGGATCCCGAGACTACTACCGACATGGTACCATGGGTTCTTGACG
```

E VIII

```
                TACGTCAGCCAGCAGGACCTGGGACCGTTTCCGTCTGGTATCCGGATTGATTACGTGCTT
2761  ---------+---------+---------+---------+---------+---------+---------+ 2820
                ATGCAGTCGGTCGTCCTGGACCCTGGCAAAGGCAGACCATAGGCCTAACTAATGCACGAA

                TACAAGGTCAGGCTCTTATTCCCGGTGTGCCTTCTCCAGTATCTTCCTTCCTCTGTCACT
2821  ---------+---------+---------+---------+---------+---------+---------+ 2880
                ATGTTCCAGTCCGAGAATAAGGGCCACACGGAAGAGGTCATAGAAGGAAGGAGACAGTGA

                AGCCCACGCTTTAGTTCAGCTACAGTCTTGGGCCACTGATGGCTAAAGAATAGAATCCTG
2881  ---------+---------+---------+---------+---------+---------+---------+ 2940
                TCGGGTGCGAAATCAAGTCGATGTCAGAACCCGGTGACTACCGATTTCTTATCTTAGGAC

                TCGGCTGGTTCTCTGGGAGAATTTAAGCTTCTCCATGTTCTTGCTCTTCCTAGGCAGTCT
2941  ---------+---------+---------+---------+---------+---------+---------+ 3000
                AGCCGACCAAGAGACCCTCTTAAATTCGAAGAGGTACAAGAACGAGAAGGATCCGTCAGA

                CTGAGTTCCACGTCTGCTGTGAGACTCTGAAAACCACTACAGGCTGTGACCCTCACAGTG
3001  ---------+---------+---------+---------+---------+---------+---------+ 3060
                GACTCAAGGTGCAGACGACACTCTGAGACTTTTGGTGATGTCCGACACTGGGAGTGTCAC
```

E IX

```
                ACAAGCCCTTCTCTGATCACGAGGCCCTCATGGCTACTTTGTATGTGAAGCACAGCCCCC
3061  ---------+---------+---------+---------+---------+---------+---------+ 3120
                TGTTCGGGAAGAGACTAGTGCTCCGGGAGTACCGATGAAACATACACTTCGTGTCGGGGG

                CTCAGGAAGACCCCTGTACTGCCTGTGGTAAGCAGCATTTCCTTTGCCCCCTCTACTTTA
3121  ---------+---------+---------+---------+---------+---------+---------+ 3180
                GAGTCCTTCTGGGGACATGACGGACACCATTCGTCGTAAAGGAAACGGGGGAGATGAAAT

                AGGCAGCCCCGCCTCCATCCTGACCCTCCCCTGCTCTACGTTCTCTCTTTTTCCAGGCCC
3181  ---------+---------+---------+---------+---------+---------+---------+ 3240
                TCCGTCGGGGCGGAGGTAGGACTGGGAGGGGACGAGATGCAAGAGAGAAAAAGGTCCGGG

                ACTGGAAAGGTCCGATTTGATCAGCGTGCTAAGGGAGGCCAGGACAGAGCTGGGGCTAGG
3241  ---------+---------+---------+---------+---------+---------+---------+ 3300
                TGACCTTTCCAGGCTAAACTAGTCGCACGATTCCCTCCGGTCCTGTCTCGACCCCGATCC
```

E X

```
                CATAGCTAAAGCTCGCTGGTGGGCTGCATTCTCTGGCTATGTGATCGTTTGGGGGCTGTC
3301  ---------+---------+---------+---------+---------+---------+---------+ 3360
                GTATCGATTTCGAGCGACCACCCGACGTAAGAGACCGATACACTAGCAAACCCCCGACAG

                CCTTCTGGTGTTGCTGTGTGTCCTGGCTGCAGGAGAAGAGGCCAGGGAAGTGGCCATCAT
3361  ---------+---------+---------+---------+---------+---------+---------+ 3420
                GGAAGACCACAACGACACACAGGACCGACGTCCTCTTCTCCGGTCCCTTCACCGGTAGTA
```

Figure 2-4

```
      CCTCTGCATACCCAGTGTGGGTCTGGTGCTGGTAGCAGGTGCAGTCTACCTCTTCCACAA
3421  ---------+---------+---------+---------+---------+---------+ 3480
      GGAGACGTATGGGTCACACCCAGACCACGACCATCGTCCACGTCAGATGGAGAAGGTGTT

      GCAGGAGGCCAAGGGCTTATGTCGGGCCCAGGCTGAGATGCTGCACGTTCTGACAAGGGA
3481  ---------+---------+---------+---------+---------+---------+ 3540
      CGTCCTCCGGTTCCCGAATACAGCCCGGGTCCGACTCTACGACGTGCAAGACTGTTCCCT

      AACGGAGACCCAGGACCGAGGCTCAGAGCCTCACCTAGCCTACTGCTTGCAGCAGGAGGG
3541  ---------+---------+---------+---------+---------+---------+ 3600
      TTGCCTCTGGGTCCTGGCTCCGAGTCTCGGAGTGGATCGGATGACGAACGTCGTCCTCCC
               stop
      GGACAGAGCTTAAGAGCTTAACAATAAAACTTGCTTGACACACTCTAGTGGCTCTACCTT
3601  ---------+---------+---------+---------+---------+---------+ 3660
      CCTGTCTCGAATTCTCGAATTGTTATTTTGAACGAACTGTGTGAGATCACCGAGATGGAA

      GTTCCTTGCAGAGGCATGATGGGAACTGAAGGTCAGTGGCCTTGTCACTGTGTGGCTTTA
3661  ---------+---------+---------+---------+---------+---------+ 3720
      CAAGGAACGTCTCCGTACTACCCTTGACTTCCAGTCACCGGAACAGTGACACACCGAAAT

      GAGCGTTGGCCTCTCACTTGCCTTTTTTGCACACTCCCGTCTCCTGCCAGCACAGAGCAT
3721  ---------+---------+---------+---------+---------+---------+ 3780
      CTCGCAACCGGAGAGTGAACGGAAAAAACGTGTGAGGGCAGAGGACGGTCGTGTCTCGTA

      AAACCCTGTTCATGGTCATAATCCTTTTATTGTAAACAACGAAGCCTCTGACTAAGCAGT
3781  ---------+---------+---------+---------+---------+---------+ 3840
      TTTGGGACAAGTACCAGTATTAGGAAAATAACATTTGTTGCTTCGGAGACTGATTCGTCA

      CCAGATGGCGGAGGTACAGCCCTTGTGATGGTGTCTTGCTTACGGGGCAGGGAGGCAGCT
3841  ---------+---------+---------+---------+---------+---------+ 3900
      GGTCTACCGCCTCCATGTCGGGAACACTACCACAGAACGAATGCCCCGTCCCTCCGTCGA

      AACCATCATCTTCTAGCCCTGGGCTCCCATCTATGCAGGCATCTCTCTGAGCCTCCGTTC
3901  ---------+---------+---------+---------+---------+---------+ 3960
      TTGGTAGTAGAAGATCGGGACCCGAGGGTAGATACGTCCGTAGAGAGACTCGGAGGCAAG

      CTCCTGGAATTGGNTCAGAGCAATCCCGCTTGGTTCACCAACCTCCAAACAGCTTCCTTA
3961  ---------+---------+---------+---------+---------+---------+ 4020
      GAGGACCTTAACCNAGTCTCGTTAGGGCGAACCAAGTGGTTGGAGGTTTGTCGAAGGAAT

      AGGACCTGGTTTCTCAAAANGGNAAGGTNCGGGCCTCCGGTCTTCAATANGTTTTCCTAA
4021  ---------+---------+---------+---------+---------+---------+ 4080
      TCCTGGACCAAAGAGTTTTNCCNTTCCANGCCCGGAGGCCAGAAGTTATNCAAAAGGATT

      AAAGGGANGAATGAAAANCCTTAAGNNCCAACAAGGGGAACCCTTGGNCCCAAAAGGGGA
4081  ---------+---------+---------+---------+---------+---------+ 4140
      TTTCCCTNCTTACTTTTNGGAATTCNNGGTTGTTCCCCTTGGGAACCNGGGTTTTCCCCT

      CCTGGGTGGTTTCCCNTTGGGGCCAAANTTATCCCAAAGGGGTCCAATTGAAGGGTTAAC
4141  ---------+---------+---------+---------+---------+---------+ 4200
      GGACCCACCAAAGGGNAACCCCGGTTTNAATAGGGTTTCCCCAGGTTAACTTCCCAATTG

      CCCCCAAAAANNACCCNTTTCCCCCGGAATTTCCAAAGGTTTNCCCCCCCCGGCAAAANC
4201  ---------+---------+---------+---------+---------+---------+ 4260
      GGGGGTTTTTNNTGGGNAAAGGGGGCCTTAAAGGTTTCCAAANGGGGGGGGCCGTTTTNG
```

Figure 2-5

```
     TCCCTTGGGGNNCCNAANCCNTGGCCCGGNCTTGGCTTTTCCCCCTTTCCCAAGNATTTC
4261 ---------+---------+---------+---------+---------+---------+ 4320
     AGGGAACCCCNNGGNTTNGGNACCGGGCCNGAACCGAAAAGGGGGAAAGGGTTCNTAAAG

     AAANNTTCCCTNGGAAANCCCCTTGNTTGGNAAAACCNAATNANGAACCANGCCAANNNT
4321 ---------+---------+---------+---------+---------+---------+ 4380
     TTTNNAAGGGANCCTTTNGGGGAACNAACCNTTTTGGNTTANTNCTTGGTNCGGTTNNNA

     TGCCAANAAACCNTTTGGGCAAAGGGGGNAAATTCANCAANGGGGNAATTGGGGAAACCC
4381 ---------+---------+---------+---------+---------+---------+ 4440
     ACGGTTNTTTGGNAAACCCGTTTCCCCCNTTTAAGTNGTTNCCCCNTTAACCCCTTTGGG

     NTGGGTTTNCCCAAAGGGCCCNAANANT
4441 ---------+---------+-------- 4468
     NACCCAAANGGGTTTCCCGGGNTTNTNA
```

Figure 2-6

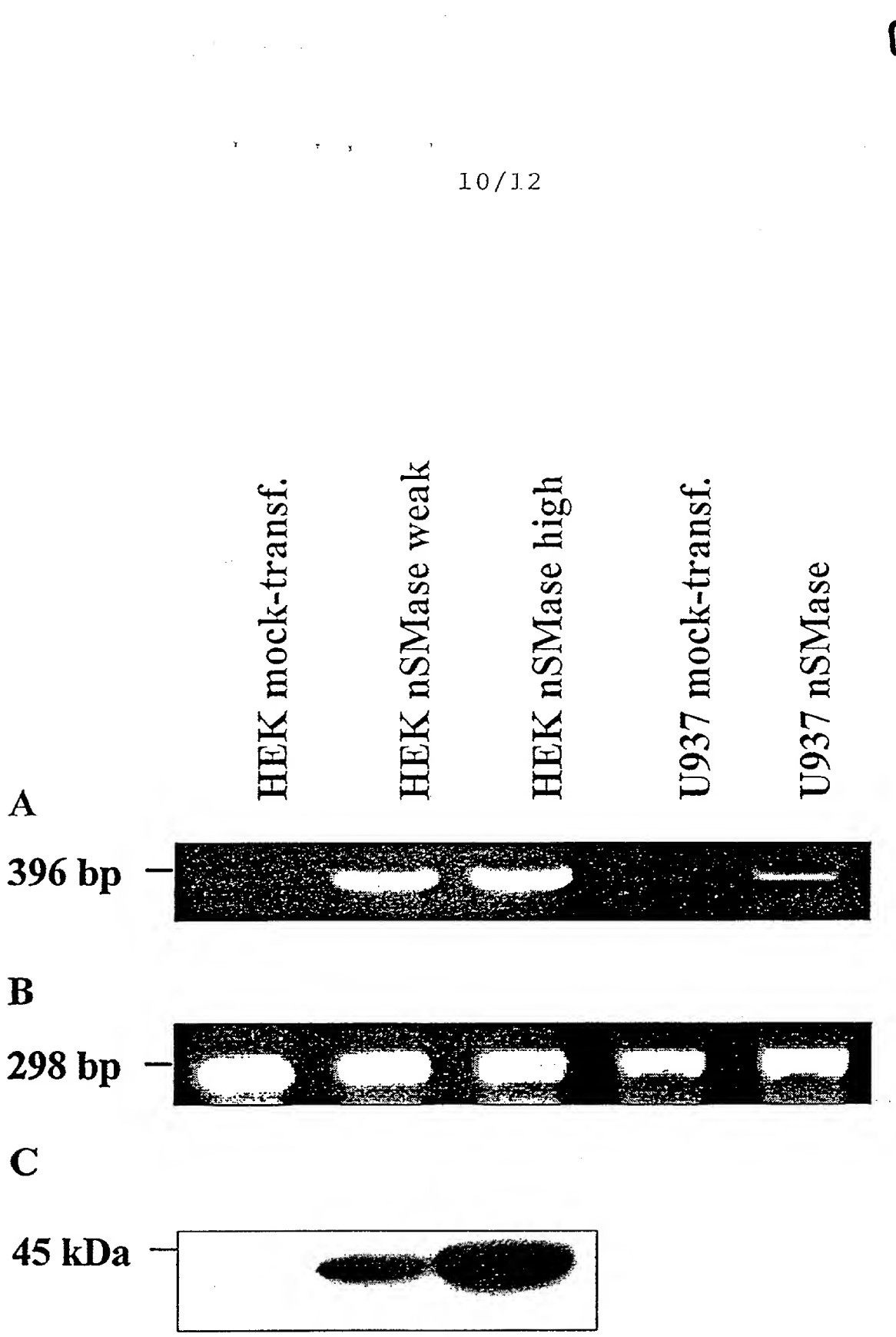
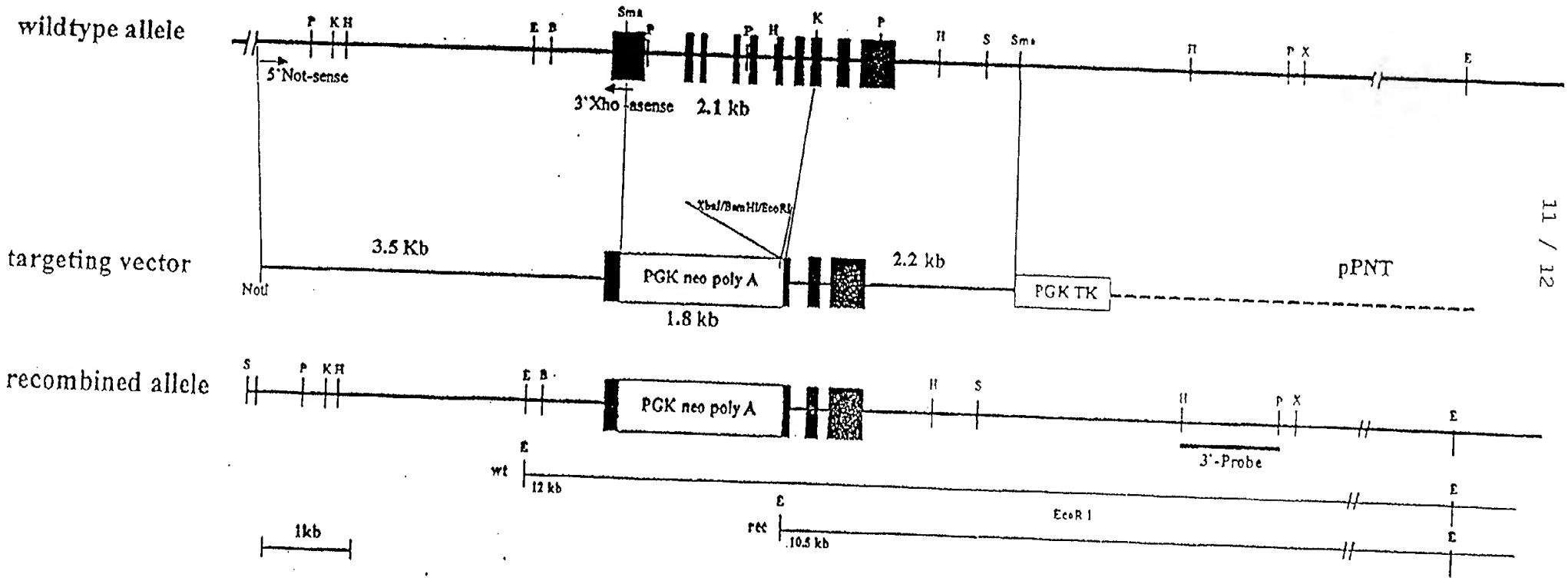

A
396 bp
HEK mock-transf.
HEK nSMase weak
HEK nSMase high
U937 mock-transf.
U937 nSMase
B
298 bp
C
45 kDa

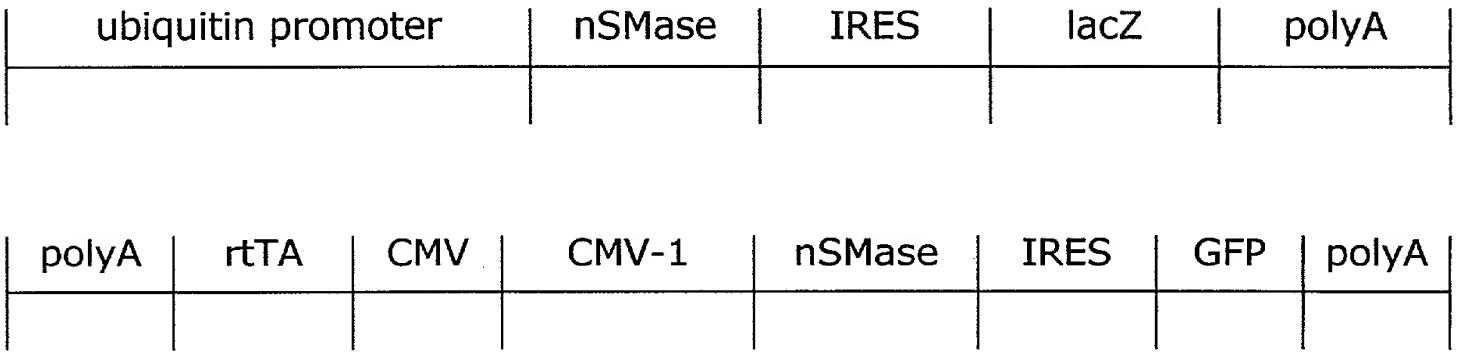
Figure 3

mnSMase "konventional" Knock Out

Figure 4



Constructs for generating transgenic mouse mutants



Ubiquitin promoter:	regulatory sequence of the ubiquitin gene, controlling a ubiquitous transcription.
nSMase:	neutral sphingomyelinase
lacZ:	lacZ, gene coding for β-galactosidase
polyA:	recognition signal for the termination of transcription and polyadenylation
CMV:	cytomegalovirus promoter of the cytomegalovirus gene, controlling a ubiquitous transcription.
rtTA:	reverse transactivator, binds to the minimal promoter and thus controls transcription. The binding properties of the transactivator are influenced by tetracyclin. The addition of tetracyclin makes the transactivator bind to the minimal promoter and starts transcription, removal of tetracyclin prevents the binding of the transactivator to the minimal promoter and prevents transcription.
CMV-1:	minimal promoter, binding of transactivator starts transcription.
IRES:	*internal ribosomal entry sequence*, viral initiation signal for translation.

Figure 5

DECLARATION AND POWER OF ATTORNEY U.S.A.

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON PRIORITY; OR PROVISIONAL APPLICATIONS

FOR ATTORNEY'S USE ONLY

ATTORNEY'S DOCKET NO. _____

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or a first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

101 NEUTRAL SPHINGOMYELINASE

102 which is described and claimed in:

[X] PCT International Application No. PCT/EP 98/05127 filed 11/08/1998

[ ] the attached specification

[ ] the specification in application _____ filed _____
(if applicable) and _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.
I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

103 104

Prior Foreign Application(s) (Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
197 34 764.9	Germany	11/08/1997	[XX] Yes [ ] No
197 58 501.9	Germany	15/10/1997	[X] Yes [ ] No
60/078,386	USA	18/03/1998	[X] Yes [ ] No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No. _____ Filing Date _____

105 I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

_____ (Application Serial No.) _____ (Filing Date) _____ (Status: patented, pending, abandoned)

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON JR. (20,851); D. DOUGLAS PRICE (24,514); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,640); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409)

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I further declare that all statements made herein of my own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 *	SIGNATURE OF INVENTOR 202 *	SIGNATURE OF INVENTOR 203 *
01/12/00 Wilhelm Stoffel	Kay Hofmann	
DATE	DATE 01/18/00	DATE 01/18/00

[ ] Additional inventors are named on separately numbered sheets attached hereto.